

Fumigant toxicity of *Carum copticum* (Apiaceae) essential oil against greenhouse aphids (*Aphis gossypii*) (Hemiptera: Aphididae) and an analysis of its constituents

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Abstract: [Aim] The cotton aphid, *Aphis gossypii* Glover, is one of the most injurious pests of fruits, vegetables and ornamental plants worldwide, both outdoor and indoor. This insect, which feeds from plant sap, produces honeydew and transmits plant viruses, and causes quantitative and qualitative damages in plants. For controlling this pest in greenhouse, plant essential oils are used as an alternative to chemical insecticides. So in this research the fumigant toxicity of *Carum copticum* L. (Apiaceae) essential oil against *A. gossypii* adults was studied. [Methods] Dry ground seeds of *C. copticum* were subjected to hydrodistillation using a modified Clevenger-type apparatus. All bioassay tests were conducted at $27 \pm 2^\circ\text{C}$, $65\% \pm 5\%$ RH and a photoperiod of 16L: 8D. This research was performed with a completely random design with 6 treatments (5 different concentrations of essential oils plus the control). Each concentration included 3 replicates and each replicate consisted of 20 adult pests. [Results] The results showed that the *C. copticum* essential oil caused significant mortality of adults 24 hours after exposure. The LC_{50} value of the essential oil against *A. gossypii* adults was $1.21 \mu\text{L/L}$ air. The mortality percentage of *A. gossypii* showed higher sensitivity to application of the essential oil. The LT_{50} value of this essential oil estimated for *A. gossypii* at the concentration of $1.21 \mu\text{L/L}$ air was 11.79 h. The fumigant toxicity of this essential oil had an ordered relationship with the concentration and time exposure. Results of GC/MS constituents showed that the essential oil consisted of 18 chemical compounds and most importantly included thymol (50.07%), gamma-terpinene (23.99%), and *p*-cymene (22.90%). [Conclusion] The results suggest that *C. copticum* essential oil has appropriate insecticidal effects on cotton aphids, which may have a promising potential to be used in the integrated pest management programs of pest insects in greenhouses.

Key words: *Aphis gossypii*; *Carum copticum*; essential oil; fumigant toxicity; gas chromatography/mass spectrometry

1 INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aleyrodidae), is scattered in many parts of the world (Kerns and Stewart, 2000). This pest has been an economically important insect pest for many years. The cotton aphid is a cosmopolitan phloem-feeding pest that causes serious damage to many crops worldwide due to direct feeding and vectoring of many plant viruses. Cotton aphids also excrete sticky honeydew which may foul some commodities and they may cause leaf yellowing or death of the host (Gildow *et al.*, 2008). If there is no control on the pest, plants will dry completely.

Considering the importance of cucurbits family, such as cucumbers, tomatoes, and beans plants, in the basket of family food and because of fresh and daily harvest of these crops, the overuse of

insecticides can create detrimental effects to the health of consumers. In any case, conventional methods in control of aphids and whiteflies in many countries, especially Iran, still are using pesticides (Palumbo *et al.*, 2001).

In order to reduce the damage caused by insects, routinely it is use of synthetic insecticides. But these compounds have undesirable effects such as damage to the ozone layer, pollution of environment, toxicity to non-target organisms, causing resistance in pests and remaining residue of chemical compounds (Ogendo *et al.*, 2003). Due to hazards and problems related in the use of chemical pesticides, they cause increased environmental limitations in these cases (Pavela *et al.*, 2010). Thus, we need to find alternative control methods environmentally friendly (Tapondjou *et al.*, 2002). Among these methods, natural products have

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attracted the attention of researchers worldwide (Kebede *et al.*, 2010).

At present, approximately 3 000 essential oils are known, 300 of which are commercially important especially for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries (Bakkali *et al.*, 2008). Some of them constitute effective alternatives or complements to synthetic compounds of the chemical industry, without showing the same secondary effects (Sosa and Tonn, 2008). Essential oils and some of their compounds are toxic to a variety of organisms including bacteria, viruses, fungi, protozoa, parasites, mites, snails and insects (Lahlou and Berrada, 2001; Papachristos and Stamopoulos, 2002; Duschatzky *et al.*, 2005; Basile *et al.*, 2006; Cavaleiro *et al.*, 2006; Liu *et al.*, 2006; Monzote *et al.*, 2006; Moon *et al.*, 2006; Priestley *et al.*, 2006; Rim and Jee, 2006; Schelz *et al.*, 2006; Soyulu *et al.*, 2006). According to pesticide residues in environment, it is better to use botanical pesticides which have no secondary effects.

Ajowan plant (*Carum copticum* L.) belongs to Apiaceae (= Umbellifera) family. It is an annual herbaceous pharmaceutical plant. Ajowan is widely distributed throughout Iran, India, Pakistan and Egypt with white flower and brownish fruit (Mohagheghzadeh *et al.*, 2007). And *C. copticum* is popularly known as ajowan. Its fruits are an important commercial product for the food/flavoring industry and spices (Pandey *et al.*, 2009).

So far, many studies have been done on insecticidal properties of botanical products of this plant. Sahaf *et al.* (2007) studied the fumigant toxicity of the essential oil of *C. copticum* against three stored-product beetles including *Callosobruchus maculatus* F., *Sitophilus oryzae* L., and *T. castaneum* H., and found that the oil has good control effect on these pests. Shojaaddini *et al.* (2008) studied the fumigant toxicity of ajowan essential oil to *Plodia interpunctella* eggs, larvae, pupae and adults and concluded that the plant essential oil has good potential for controlling Indian flour moth in stored products. The results of researchers showed the lethal properties existing in this oil.

Insecticidal effects of different essential oils on cotton aphids have been slightly researched. For example, Ebrahimi *et al.* (2013) tested the insecticidal activity of three plant essential oils, *i. e.*, azadirachtin (*Azadirachta indica* A. Juss.), eucalyptus (*Eucalyptus camaldulensis* Dehnh.) and laurel (*Laurus nobilis* L.) on cotton aphid (*A. gossypii*), and concluded that azadirachtin and eucalyptus are more lethal than laurel essential oil.

No study has been reported previously concerning the activity of the essential oil as fumigants against this pest. The present study was conducted to determine the efficiency of essential oil from *C. copticum* as a fumigant in management of the important greenhouse pest *A. gossypii*.

2 MATERIALS AND METHODS

2.1 Collection and drying of the plant sample

Seeds of ajowan were collected from Urmia in Western Azerbaijan province. And after transmission to laboratory in dark condition and suitable ventilation, the seeds were dried and maintained at the temperature of -24°C .

2.2 Extraction of essential oil

The ajowan seeds were air-dried in the shade at room temperature ($26 - 28^{\circ}\text{C}$) and the essential oil was isolated by hydro distillation method using a Clevenger apparatus. Conditions of extraction were: 50 g of air-dried sample, 1: 6 in water (w/v), 2 h distillation. Essential oil was stored in refrigerator at 4°C and was kept away from light. Compounds of essential oil were determined by method of gas chromatography in biotechnology in Institution of Urmia University.

2.3 Rearing of insects

Cotton aphids were reared on tomatoes plants, in temperature condition $27 \pm 2^{\circ}\text{C}$, $65\% \pm 5\%$ RH and photoperiod of 16L: 8D in greenhouse.

2.4 Creating *A. gossypii* aphids of the same biological age

By method of Ebrahimi (2013), 20 adult insects were put on each leaf. After 1 day, adult insects were picked up from each leaf and the larvae were permitted to rear in the same condition to reach the same biological age. After 7 – 8 days larvae reached to adult stage.

2.5 Determination of the median lethal concentration (LC_{50})

For determining the LC_{50} , different doses of essential oil were shed at three replications on filter paper, into a 305 mL glass dishes which consisted of 20 adult insects with nutrition substance (cucumber leaf) and glass was sealed with parafilm to prevent any loss of essential oil. Mortality was recorded after 24 hour past from exposure to different concentrations. These insects incapable of moving after a slight touch with a fine brush were considered as dead (Choi *et al.*, 2003).

2.6 Determination of the median lethal time (LT_{50})

The glass (305 mL) contained 20 adults (1 – 2 day-old) via nutrition substance (cucumber leaf).

The LT₅₀ doses were dropped on filter paper in plug of glass and sealed with parafilm, and then insect mortality in successive time was calculated. In this experiment, the insects with a hot needle closer to their legs and antennae not moving were considered dead. The experiment was done in three replicates.

2.7 Gas chromatography/mass spectrometry

The constituents of *C. copticum* essential oil were analyzed by gas chromatography mass spectrometry (GC-MS) (Thermo-UFM). The GS conditions were as follows; capillary column pH 5 (10 m × 0.1 mm, film thickness 0.4 μm); helium as a carrier gas (0.5 mL/min); oven temperature program, initially 60°C rising to 285°C (80°C/min, 3 min); injector and detector temperature of 280°C. The identification of individual compounds was based on comparison of their relative retention time with those of authentic samples on a capillary column, and by matching their mass spectra of peaks with those obtained from authentic samples and published data.

2.8 Data analysis

The mortality after 24 hours of treatment was surveyed, the number of dead insects in treatments and the control was counted and mortality percentage was calculated according to Abbott’s formula (Abbott, 1925). The archived data were analyzed with SPSS (V. 19) program (SPSS 2011). After 24 h the LC₅₀ value was calculated for oil. For the mean comparison about effect value of different concentrations of essential oil, we used Tukey’s test at 1% statistical confidence level of 99%.

3 RESULTS

3.1 The LC₅₀ and LT₅₀ of *Carum copticum* essential oil against *A. gossypii* adults

The results of bioassays to determine fumigant toxicity of ajowan plant essential oil showed that it had a strong toxicity against the greenhouse aphid. The LC₅₀ value was estimated 24 hours after exposure for greenhouse aphid 1.21 μL /L air (Table 1).

Table 1 LC ₅₀ and LC ₉₅ values of <i>Carum copticum</i> essential oil against <i>Aphis gossypii</i> adults during 24 hours						
Insect species	Insect number	χ ² (df)	Intercept (a) + 5	Slope (mean ± SE)	LC ₅₀ (μL/L air) ¹	LC ₉₅ (μL/L air) ¹
<i>A. gossypii</i>	300	3.69 (3)	4.75	2.97 ± 0.31	1.21 (1.06 – 1.38)	4.33 (3.40 – 6.14)

¹ Lower and upper 95% fiducially limits are shown in parenthesis.

These values were dependent on oil concentration and exposure time (Figs. 1 and 2). Given the results of this study, the obtained *F* value with 5 degrees of freedom was 78.541, suggesting significant difference between treatments in 1% level probability (Table 2).

Table 2 ANOVA analysis of the effect of <i>Carum copticum</i> essential oil at various concentrations against <i>Aphis gossypii</i> adults					
Source	df	Sum of squares	Mean square	<i>F</i>	Sig.
Concentration	5	11 151.557	2 230.311	78.541	0.000
Error	12	340.759	28.397		
Total	17	11 492.316			

Average comparison was done, showing that the 5th concentration by 60 lethal percentage in A group has located and the highest lethal percentage has been created. Fourth concentration in A, B groups and third dose in B, C groups and second concentration in C group have located. First concentration in D group has located and lethal percentage 10 has been created (Table 3).

Table 3 Comparison of means of effect values of <i>Carum copticum</i> essential oil against <i>Aphis gossypii</i> adults	
Concentration (μL/L air)	Mortality (%)
0.33	10.64 ± 5.28 D
0.66	25.93 ± 1.92 C
1.31	35.08 ± 1.92 BC
1.97	50.99 ± 3.66 AB
2.62	60.95 ± 2.85 A

Means in a column followed by different capital letters are significantly different by Tukey’s test at the 1% level.

The results of bioassay tests showed that at the LC₅₀ concentration (1.21 μL /L air), the LT₅₀ value of the oil against the cotton aphid was 11.79 (Table 4).

Given the results of study from data of time analyze one-way ANOVA, obtained *F* value, suggesting significant difference between treatments in 1% level probability (Table 5).

Table 4 LT ₅₀ value of <i>Carum copticum</i> essential oil against <i>Aphis gossypii</i> adults					
Insect species	Insect number	χ ² (df)	Intercept (a) + 5	Slope (mean ± SE)	LT ₅₀ (μL/L air)
<i>A. gossypii</i>	300	1.81 (3)	3.49	1.41 ± 0.22	11.79 (9.21 – 15.51)

Table 5 ANOVA analysis of the effect of *Carum copticum* essential oil against *Aphis gossypii* adults in different time

Source	df	Sum of squares	Mean square	F	Sig.
Concentration	5	6 433.098	1 286.620	150.145	0.000
Error	12	102.830	8.569		
Total	7	6 535.928			

Mean comparison was performed, showing that 5th time by 57 lethal percentage in A group has located and the highest lethal percentage has been created. Fourth time in A, B groups, and third time in B, C groups and second time in C, D groups have located. First concentration in D group has located and lethal percentage 24 has been created (Table 6). That in first time (120 min) 24 percent lethal caused and during time change creates mortality increase.

Table 6 Comparison of means of effect values of exposure time of *Carum copticum* essential oil against *Aphis gossypii* adults

Time (min)	Mortality (%)
120	24.05 ± 1.26 D
420	35.17 ± 2.72 CD
720	43.09 ± 0.96 BC
1 080	50.79 ± 1.69 AB
1 440	57.91 ± 2.09 A

Means in column followed by different capital letters are significantly different by Tukey test at the 1% level.

3.2 Gas chromatography/mass spectrometry determination of the constituents of the *C. copticum* essential oil

According to the gas chromatographic analysis of *C. copticum* essential oil (GC/MS), it was determined that the oil consists of 18 chemical compounds and most importantly included: thymol (50.07%), gama-terpinene (23.99%), and *p*-cymene (22.90%) (Table 7). The analysis of substances existence in *C. copticum* essential oil was given (Table 7). That the analysis of the results of this research was conducted on plant ajowan by Moazeni *et al.* (2012) is fully consistent with substances existence in the plant.

Among the major monoterpenoids of the essential oil, it was reported that gama-terpinene and thymol are the most active constituents against adults of the rice weevil, *S. oryzae* (Erler, 2007). The rapid action against Indian meal moth may be indicative of a neurotoxic mode of action. There is evidence for the octopaminergic system as a target for some monoterpenoids (Enan, 2001). Research results indicate that mortality of insects was caused by monoterpene because of its inhibitory effect on the acetylcholinesterase enzyme (Houghton *et al.*, 2006).

Table 7 Chemical composition of the *Carum copticum* essential oil identified by gas chromatography-mass spectroscopy

No.	Component	Proportion (%)
1	Alpha-thugin	0.32
2	Alpha-pinene	0.11
3	1,8-Cineole	0.50
4	Sabinene	0.19
5	Beta-pinene	0.42
6	Myrcene	0.51
7	Alpha-perpinene	0.02
8	<i>p</i> -Cymene	22.90
9	<i>Cis</i> -sabinene hydrate	0.04
10	Gama-terpinene	23.99
11	Linalool	0.01
12	<i>Trans</i> -sabinene hydrate	0.03
13	Thymol	50.07
14	Cyclocitral	0.13
15	Aipha-terpineol	0.09
16	Terpinen-4-ol	0.11
17	Carvacrol	0.13
18	Ocimene	0.04

4 DISCUSSIONS

Experimental results indicate that the essential oil of *C. copticum* have a strong lethal effect on the cotton aphid. Toxicological properties of compounds in the essential oil to different pests have been reported in scientific resources and can be in various forms of respiratory, gastroenterology and contact toxicity against insects.

In other experiments, Soliman (2006) studied the insecticidal effect of *Artemisia herba-alba* (Asso) and *Artemisia monosperma* (Delile) essential oils on three species of sucking pests such as cotton aphid (*A. gossypii* Glover), whitefly (*Bemisia tabaci* Gennadius) and onion thrips (*Thrips tabaci* Lindeman) in greenhouse conditions and the results of these tests showed that the essential oils have higher lethal effect on *T. tabaci* and *A. gossypii* than on *B. tabaci*. This study has shown the lethal effect of the essential oil from *C. copticum* on *B. tabaci* which belongs to Aleyrodidae family. There is similar sensitivity between members of a family. Then our study is near to this research.

Ebrahimi *et al.* (2013) study showed that Azadirachtin (*Azadirachta indica* Adr. Juss.), eucalyptus (*Eucalyptus camaldulensis* Dehnh.) and laurel (*Laurus nobilis* L.) had significant efficacy on mortality of *A. gossypii*, for this reason, azadirachtin and eucalyptus essential oils have been more killing

than laurel oil. This study agrees with the results of these researches showing high sensitivity of *A. gossypii* to application of essential oils. In the studies on the control effect of some essential oils that might be useful in the control of foxglove aphid, *Aulacorthum solani* Kalt. (Hemiptera: Aphididae) on eggplant plant (*Solanum melongena* L.), Górski and Tomczak (2010) concluded that the use of these essential oils caused 100% mortality in the population of this pest. This study agrees with the results of Górski and Tomczak (2010) study about sensitivity of Aphididae family to application of natural essential oils. Research of Sahaf *et al.* (2007) concluded insecticidal activity of *C. copticum* against *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (L.). In other experiments, Sahaf and Moharramipour (2008) conducted the sublethal effects of two species of *Carum copticum* and *Vitex pseudo-negundo* essential oils on eggs, larvae and adults of *Callosobruchus maculatus*, showing that the *C. copticum* essential oil had higher toxicity than *V. pseudo-negundo* essential oil on pest. Sahaf *et al.* (2007) and Sahaf and Moharramipour (2008) demonstrated insecticidal effect of *C. copticum* that agree with this study in case lethal properties of *C. copticum* essential oil. Isman (2000) demonstrated the acaricidal effect of *C. copticum* essential oil against two spitted mite (*Tetranychus urticae* Koch). These results are consistent with the results of the present study. Research of Shojaaddini *et al.* (2008) showed the fumigant toxicity of *C. copticum* essential oil to Indian flour moth (*Plodia interpunctella*), and this essential oil has a noticeable effect in control of different developmental stages (egg, larva, pupa and adult) of *P. interpunctella*. According to Rani and Khullar (2004), *C. copticum* essential oil has antimicrobial properties against *Salmonella typhi* (Schroeter), which is resistant to multiple drugs. And also this essential oil had nematicidal properties (Jabbar *et al.*, 2006; Lateef *et al.*, 2006; Park *et al.*, 2007). Lethal effect of *C. copticum* has been demonstrated by above researchers which corresponded with the results of our study.

From family Apiaceae can *C. carvi* be noted. According to Pavela (2011). its essential oil was demonstrated with insecticidal and repellency activity on pollen beetle adult (*Meligethes aeneus* Fabricius). It was also reported with repellency effect on mosquitoes (Pavela *et al.*, 2009; Nerio *et al.*, 2010). These researches showed different effects of *C. carvi*, indicating the sensitivity of insects to the application of *C. carvi* essential oil.

Arouiee *et al.* (2009) investigated the insecticidal activity of the essential oils of three different medicinal plants (caraway, fennel and rosemary) for the control of *T. vaporariorum* and the results indicated that the most effective essential oils from caraway (*Carum carvi* L.) and fennel (*Foeniculum vulgare* Mill.) are active at the concentrations of 7.5 and 5 $\mu\text{g/mL}$, respectively, ratio rosemary oil (*Rosmarinus officinalis*). This study agrees with the results of Arouiee *et al.* (2009) for the high lethal effect of mentioned essential oils for control pest. This plant belongs to Apiaceae family. There is similar effect between members of a family. Then *C. copticum* essential oil have lethal and repellency effect which can be seen in our study.

Consumption of botanical oils caused decreased use of chemical toxins and as a result caused decreased damage of insect pests. Essential oils of medicinal plants are recommended as a combination of environmental friendliness and low probability of pest resistance to plant oils in controlling pest population. The results and similar studies show that essential oils can be used as potential sources for pest control in greenhouses. In this study, for the first time the fumigant toxicity of *C. copticum* to *A. gossypii* was studied. The results of this experiment show that this essential oil has good control effect on *A. gossypii* adults.

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印度藏茴香植物精油对温室棉蚜的熏蒸毒性及其成分分析

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摘要:【目的】棉蚜 *Aphis gossypii* 是世界各地室内和室外果树、蔬菜和观赏植物上最具危害性的害虫之一。这一害虫取食植物汁液,产生蜜露,传播植物病毒,对植物从质和量上产生破坏。为了控制温室中的这一害虫,植物精油可用作化学农药的替代药物。本实验研究了印度藏茴香 *Carum copticum* 植物精油对棉蚜成虫的熏蒸毒性。【方法】将研磨的印度藏茴香干种子用改良的挥发油提取器(Clevenger-type apparatus)进行水蒸馏。所有生物测定均在 27 ± 2℃,相对湿度 65% ± 5% 和光周期 16L: 8D 条件下进行。研究采用完全随机设计,6 个处理(5 个不同浓度的精油加对照)。每一浓度 3 次重复,每一重复 20 头成虫。【结果】结果表明,棉蚜成蚜接触印度藏茴香精油 24 h 后出现明显的死亡。该精油对棉蚜成蚜的致死中浓度(LC₅₀)为 1.21 μL/L 空气。棉蚜成蚜的死亡百分率对精油的施用表现出较高的敏感性。精油浓度为 1.21 μL/L 空气时,估计的棉蚜的致死中时(LT₅₀)为 11.79 h。这一精油的熏蒸毒性与浓度和接触时间之间具有一定的相关性。GC/MS 组分分析结果表明,该精油由 18 种化合物组成,最重要的是一些化合物引起了熏蒸毒性,如麝香草酚(占 50.07%)、γ-萜品烯(占 23.99%)和对异丙基苯甲烷(占 22.90%)。【结论】本研究结果表明印度藏茴香植物精油对棉蚜具有较好的杀虫效果。印度藏茴香精油对棉蚜产生较大的影响,由于它具有较高的熏蒸毒性潜力,因此可在温室中用于这一害虫的综合治理。

关键词: 棉蚜; 印度藏茴香; 精油; 熏蒸毒性; 气相色谱-质谱

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